Letter

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Discovery of Potent and Selective Tricyclic Inhibitors of Bruton's Tyrosine Kinase with Improved Drug-like Properties

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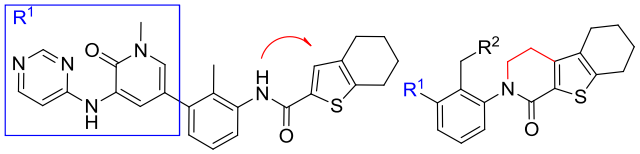
ABSTRACT: In our continued effort to discover and develop best-in-class Bruton's tyrosine kinase (Btk) inhibitors for the treatment of B-cell lymphomas, rheumatoid arthritis and systemic lupus erythematosus, we devised a series of novel tricyclic compounds that improved upon the drug-like properties of our previous chemical matter. Compounds exemplified by **G-744** are highly potent, selective for Btk, metabolically stable, well tolerated and efficacious in an animal model of arthritis.

Bruton's tyrosine kinase (Btk) plays a critical role in the function of B cells and myeloid cells, making it an attractive target for the treatment of immunological disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS).[1-4] as well as B-cell lymphomas.[5] Accordingly, there have been significant efforts from the pharmaceutical community toward identifying Btk inhibitors for clinical evaluation.[6-14] Of these, the most advanced compound to date is ibrutinib, recently approved for treatment of Mantle Cell Lymphoma (MCL), chronic lymphocytic leukemia (CLL), and Waldenstrom's macroglobulinemia and under evaluation in additional indications.[15]

We have previously reported on the discovery of several series of novel and potent Btk inhibitors that possess exquisite selectivity for Btk over other kinases.[9-11,16] Their high potency and selectivity stems from the ability of compounds such as **1** to create an induced binding fit in the protein via a rearrangement of the activation loop.[3] Other kinases, including members of the tyrosine kinase family cannot rearrange in the exact same way due to differences in the amino acid sequence within this loop.[11] The tetrahydrobenzothiophene moiety within **1**, held rigidly in place by other contacts the inhibitor makes with the protein, presents a hydrophobic surface that "attracts and sequesters" Y551, ultimately creating a lipophilic specificity pocket ("H3"). A common feature in this series is the incorporation of a secondary amide that tethers the distal bicyclic ring to the central benzene. We set out to address metabolism and permeability liabilities associated with the amide moiety.

Table 1: Improved properties of tricyclic H3 Btk inhibitors



1 ⟹ **2a** R^2 = H; **2b** R^2 = OH

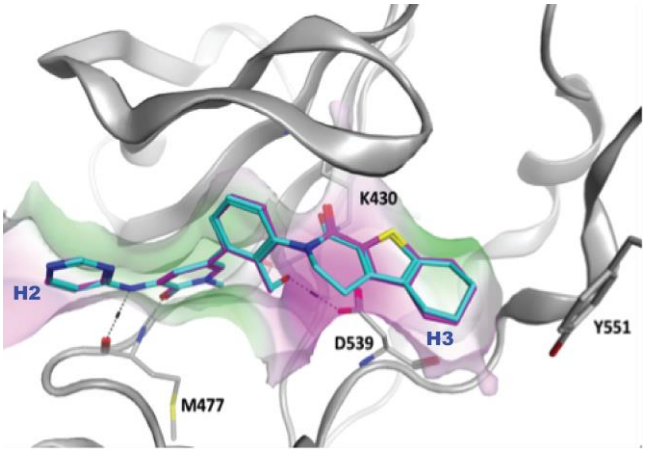
ID	IC_{50} (μM)[a]		LLE[b]	hPPB[c]	Rat	
	Btk	CD86			CL[d]	F%[e]
1	0.042	0.74	3.6	99%	2	45%
2a	0.011	--	4.2	99%	--	--
2b	0.001	0.087	5.8	97%	2	90%

[a]*Assay protocols in Supporting Information (SI), n ≥ 2.* [b]*LLE = pIC_{50}(Btk) – cgLogD.* [c]*Human plasma protein binding.* [d]*Total clearance (mL/min/kg) at 1 mg/kg i.v. dose formulated using a mixture of EtOH/Cremophor/water for* ***1*** *(solution) or PEG400/EtOH/water for* ***2b*** *(solutions).* [e]*F% = oral bioavailability after a 5 mg/kg oral dose (n=3) formulated using a mixture of EtOH and Cremophor for* ***1*** *(solution) or PEG400/EtOH/Tween80/water for* ***2b*** *(suspension).*

In particular, we hypothesized that molecules with an amide tethered back onto the tetrahydrobenzothiophene, such as **2a**, would offer a molecule with one less exposed N-H donor and reduce the number of rotatable bonds, potentially improving potency and permeability. Crystal structures of related compounds bound to Btk indicated the amide N-H was not interacting with the protein.[3] Additionally, we incorporated a hydroxyl group in the central benzene ring that was well positioned to interact with a nearby K430 and D539 residues, with the hope of improv-

ing binding affinity. Table 1 shows the potencies of these compounds for Btk in a biochemical assay, as well as their potencies in an *in vitro* mouse splenocyte CD86 cell based assay that was used as a downstream pharmacodynamic marker for Btk activity (assay protocols in SI). Pleasingly, the newly generated tricyclic compound **2a** was roughly 4-fold more potent against Btk than the uncyclized precursor **1**. Compound **2b**, which includes a hydroxyl group on the central benzene ring, has a ~10-fold increase in Btk binding potency than **2a** and is roughly 40-fold more potent than compound **1**. With the improved potency and/or decreasing lipophilicity, the lipophilic ligand efficiency (LLE) increased sequentially from **1** (3.6) to **2a** (4.2) to **2b** (5.8), indicating enhanced drug-like properties. Cellular potency of compound **2b** (IC_{50} = 0.087 μM), as measured by inhibition of CD86 surface expression on B cells, is 8-fold higher than **1** (IC_{50} = 0.74 μM). Additionally, **2b** is 2-fold more orally bioavailable (90%) than **1** (45%) and has similarly low clearance in rat.

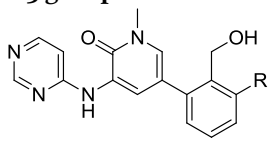
Figure 1: Superposition of modeled structures of **1** (magenta) and **2b** (cyan) in the Btk catalytic domain. H-bonds to the protein are shown as black cylinders with dashed lines. The active site surface is shown color coded by lipophilicity (green, nonpolar; purple, polar).

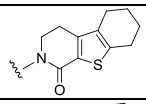
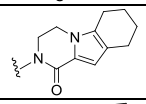
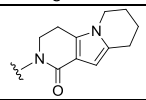
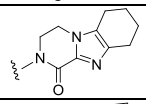
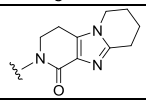
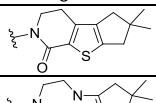
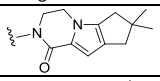
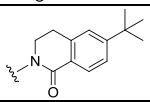
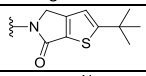
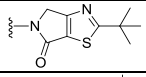
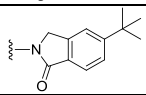
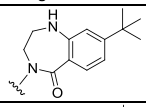
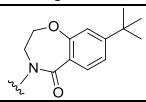


Modeling of **1** and **2b** in the Btk catalytic domain is shown in Figure 1. The tricyclic system did not alter the overall predicted ligand binding mode, and preserved the interactions at the kinase selectivity H3 pocket with the neighboring hydrophobic residues. Specific interactions with Y551 and K430 were maintained. The hydroxymethyl on the central benzene ring projected into a water-filled cavity, and formed H-bonds with K430 and D539.

The success of our initial attempts at creating improved Btk inhibitors by the employment of a tricyclic moiety encouraged us to explore additional tricycles at this position as well as carefully designed bicycles. Table 2 shows a selected subset of such compounds. Compounds **2b**-**6** are examples of inhibitors that contain distal 6-5-6 tricyclic ring systems. Since the H3 site is lipophilic, with nonpolar residues flanking much of this pocket, it is not surprising that the most potent compounds **2b** and **3** (Btk IC_{50} = 0.001, 0.006 μM, respectively) contain all-carbon fused cyclohexane rings.

Table 2: SAR for H3 groups



ID	R	IC_{50} (μM)[a]		S (μM)[b]
		Btk	CD86	
2b		0.001	0.087	2
3		0.006	0.2	49
4		0.081	0.48	85
5		2.6	48.6	36
6		16.7	>50	124
7 G-744		0.002	0.064	1.4
8		0.002	0.057	1
9		-	0.051	2
10		0.003	0.193	2
11		0.139	NT	56
12		0.003	1.2	8
13		0.004	0.171	15
14		0.005	0.23	2

[a]*Assay protocol in SI, n ≥ 2.* [b]*Kinetic solubility was measured at pH7.4.*[17]

Compound **4**, with a heteroatom in the right hand saturated ring, displayed reduced potency (Btk IC_{50} = 0.081 μM), while **5** and **6** were much less potent, presumably due to the reduced lipophilicity of the tricycle. Modeling indicated that there was an opportunity to further flesh out the H3 pocket and that gem dimethyl substituted 6-5-5 systems might offer some advantages by occupying more of the lipophilic H3 pocket. The resulting 6-5-5 compounds **7**

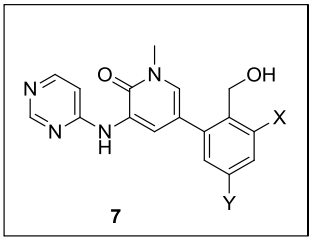
and **8** of **2b** and **3** respectively were equi- to slightly more potent against Btk (IC_{50} = 0.002 μM), and both had improved cell potencies.

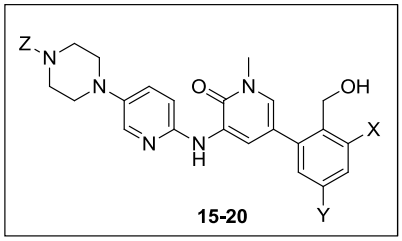
We also examined bicyclic 6-6 fused (**9**), 5-5 fused (**10**-**11**), and 5-6 fused (**12**) ring systems containing appropriately placed *t*-butyl groups to extend into the H3 pocket. Among this group, the 5-5, 5-6 fused **10** and **12** had the highest enzyme binding potency (Btk IC_{50}'s = 0.003 μM) but were relatively less active in the CD86 cellular assay.

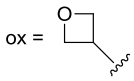
Finally, we surveyed 7-6 ring systems (**13** and **14**). These compounds were less appealing due to their decreased biochemical and cell potencies. The cell potencies of all compounds mostly tracked with biochemical potencies, with the Btk/CD86 IC_{50} ratio ranging from 6- (**4**) to 64- (**10**) fold. The only major outlier was the bicyclic compound **12** which had a cell shift of 400-fold. Presumably, this shift was due to differences in plasma protein binding and cell permeability of this compound. Other researchers have reported on a variety of bicyclic H3 moieties within alternate series,[18-20] however we are the first group to describe highly potent and Btk-selective tricyclic 6-5-6 and 6-5-5 compounds.

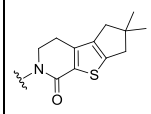
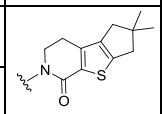
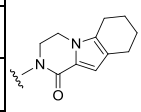
With a variety of novel tricyclic compounds in hand that had excellent potency for Btk, we focused our efforts on designing compounds with improved physiochemical properties. As demonstrated in Table 2, although compounds **2b**, **7**, **8**, and **9** had the best cellular potency (CD86 IC_{50} < 100 nM), they unfortunately all had low kinetic solubility (< 2 μM) at physiological pH. Kinetic solubility as measured in an internal high throughput assay[17] was used as an initial gauge of intrinsic solubility. Low solubility is a primary cause of reduced oral bioavailability of the crystalline materials preferred for clinical development, and often presents formulation challenges in dose escalations for safety studies. This was indeed the case for many compounds in this chemical series. To find compounds with improved solubility, modifications of the left-hand portion of the inhibitor that extends into a partially solvent exposed area of the protein region ("H2") were explored and found to be well tolerated. Of the many compounds generated,[21] we determined that compounds with a substituted pyridinopiperazine group (Table 3), as opposed to the pyrimidine of the previous compounds (Table 2), offered an exciting new sub-series with much improved kinetic solubility.

Table 3: Combination of the best H2 and H3 moieties for optimal potency and drug-like properties







ID	X	Y	Z	IC_{50} (μM)[a]			HHep/ RHep [b]	Sol. (μM)[c]	Perm.[d]	Rat	
				Btk	CD86	WB CD69				CL[e]	F%[f]
7		H	n/a	0.002	0.064	0.087	11 / <10	1.4	MOD	16	41
15		H	Me	0.003	0.025	-	<6.2 / <10	18	-	29	37
16		H	ox	0.004	0.006	0.089	8.8 / <10	1	MOD	-	-
17		H	Me	0.004	0.107	0.045	10 / 12	115	MOD	40	20
18		H	ox	0.008	0.137	0.069	<6 / <10	99	MOD	14	46
19		F	Me	0.002	0.022	0.029	<6.2 / <10.1	71	MOD	37	52
20		F	ox	0.004	0.010	0.035	<6 / <10	73	HIGH	20	27

[a]*Assay protocol in SI, n ≥ 2.* [b]*Projected hepatic clearance using human or rat hepatocytes.* [c]*Kinetic solubility was measured at pH7.4;*[17] [d]*Measured permeability using Madin-Darby Canine Kidney (MDCK) Epithelial cell lines, A to B; MOD: 1-10 (10^{-6} cm/s) and HIGH: > 10 (10^{-6} cm/s).* [e]*Total clearance (mL/min/kg) at 1 mg/kg i.v. dose formulated using a mixture of PEG400/ethanol/water for **7**, **15**, and **17-20** (solution);* [f]*F% = oral bioavailability after 5 mg/kg oral dose (n=3) formulated using a mixture of PEG400/ethanol/Tween80/water for **7** (suspension), PEG400/ethanol/water for **17** (suspension) and **15**, **18-20** (solution).*

A small subset of compounds containing combinations of the best H2 and H3 groups are highlighted in Table 3. Installing a tertiary basic amine containing left-hand H2, which is primarily protonated at neutral pH, indeed helped improve the solubility from 1.4 μM (**7**) to 18 μM (**15**). Reducing the basicity from calculated pK_a of 7.8 (**15**) to 6.3 (**16**, substituted with an oxetane)[22] abrogated the improved solubility. This prompted us to focus on H3 groups with higher polarity than that of **7**. Gratifyingly, all three compounds containing a more polar tetrahydroindole H3 group (**17-20**) maintained high kinetic solubility regardless of the amine basicity. However, the moderate CD86 cellular potencies of **17** and **18** (IC_{50}'s of 0.107 and 0.137 μM, respectively) mirrored that of the 0.2 μM potency seen for **3,** which bears the same polar H3 group. A potency breakthrough was realized when a single fluorine atom was added to the middle linker benzene ring (**19-20**). This fluorine is well positioned to engage the backbone carbonyl carbon of G409 in a dipole-dipole interaction (Figure s1 in SI), further stabilizing the binding orientation of these inhibitors in the Btk catalytic domain. The resultant CD86 cellular potency improved 5-fold and 13-fold for the two pairs **17**→**19** and **18**→**20** respectively.

All compounds in table 3, except **17**, were low to moderately cleared and had reasonable bioavailability in *in vivo* rat PK studies. *In vitro* hepatic clearance represented by RHEP data underestimated the *in vivo* clearance, however the relative stability trend was consistent from *in vitro* to *in vivo* studies. It is also pleasing to see that human hepatic clearance ranged from low to moderate. Given the totality of data including safety assessment (not discussed here), compounds **7** (**G-744**) and **20** rose to the top of the list with the most balanced overall profile.

In addition to potency and drug-like properties, a prominent goal of developing a clinically viable kinase inhibitor lies in controlling selectivity. Poor selectivity may have a profound effect on drug safety, especially for non-oncology indications where chronic dosing requires an exquisitely clean safety profile. Therefore both **G-744** and **20** were profiled against a panel of 285 active recombinant human kinases. In particular, **G-744** demonstrated >1000-fold Btk biochemical selectivity against all kinases tested except for EphA7 and Fgr, against which it still showed robust selectivity of 428-fold and 868-fold, respectively (Figure 3s in SI). Due to **G-744**'s superb kinase selectivity (superior to **20**), we realized it could be an excellent tool molecule to probe the biology of Btk as the results would not be confounded by off-target activity and the need for interpretation. Thus, we performed a full characterization of this molecule, with key data summarized in Table 4. In addition to preventing cellular functions in murine B-cells such as B-cell receptor (BCR)-mediated CD86 induction with an EC_{50} of 64 nM, **G-744** also inhibited BCR-stimulated B-cell proliferation in human B-cells (EC_{50} = 22 nM). In human moncocytes, production of the inflammatory cytokine TNFα following activation with immune-complexes was abrogated by **G-744** (EC_{50} = 33 nM). In human whole blood, **G-744** demonstrated potent inhibition of BCR-stimulated CD69 expression on B-cells with an EC_{50} of 87 nM.

Table 4: Additional **G-744** potency data (mean ± SEM)

Assay	*$K_{i,app}$ or EC_{50} (nM)*
Btk biochemical, ($K_{i,app}$)	*1.28 ± 0.13 [n = 3]*
Mouse splenocyte B cell CD86	*55 and 75 [n = 2]*
Human Whole Blood B cell CD69	*87 ± 30 [n = 11]*
Human B cell proliferation	*22 ± 3 [n = 17]*
Human monocyte TNFα production	*33 ± 6 [n = 12]*

In pharmacokinetic experiments, **G-744** exhibited low to moderate clearance in four preclinical species (Table 5). Sufficient oral exposures were achievable using a crystalline formulation in both mouse and rat despite low kinetic solubility.

Table 5: Preclinical DMPK profiling of **G-744**

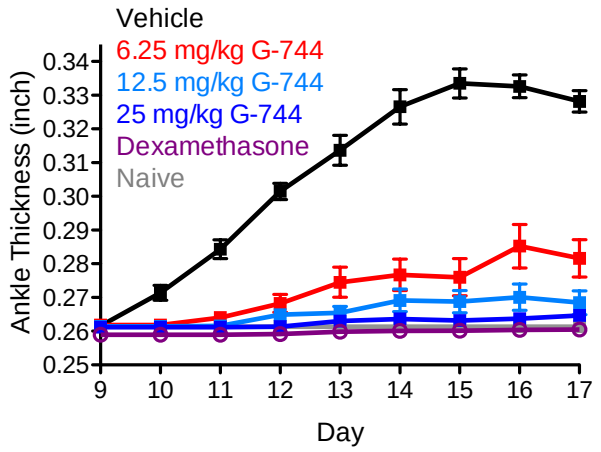
Species	**In vitro**		**In vivo**	
	LM CL_{hep}[a]	**Hep CL_{hep}[b]**	**CL[c]**	**F%[d]**
mouse	35	2	22	45[e] - 77[f]
rat	18	3	16	23[e] - 37[f]
dog	15	1	7	27[f]
cyno	18	31	17	--
human	8	11	--	--

[a]*Projected hepatic clearance using liver microsomes (mL/min/kg).* [b]*Projected hepatic clearance using hepatocytes (mL/min/kg).* [c]*Total clearance in vivo (mL/min/kg).* [d]*F% = oral bioavailability after 5 (amorphous) or 100 mg/kg (crystalline) dose in mouse; 5 (amorphous) or 100 mg/kg (crystalline) dose in rat; 5 mg/kg dose in dog (n = 3).* [e]*Methylcellulose/Tween80/water suspension of crystalline free base material.* [f]*Hydroxypropylmethethylcellulose/Na citrate/water pH 3 suspension of amorphous material*

Given the favorable DMPK profile of **G-744** in rat, we examined its efficacy in the developing collagen-induced arthritis (CIA) model in Lewis rat.[3,23] Oral dosing with **G-744** at 6.25, 12.5 and 25 mg/kg b.i.d. overall maintained plasma concentrations above the IC_{50}, IC_{70} and IC_{90}, respectively, for inhibition of Btk Y223 phosphorylation in whole blood (Figure s2 in SI). As shown in Figure 2, all three doses resulted in a significant dose-dependent inhibition of ankle thickness between day 10 and day 17 (onset of increase in ankle diameter on day 9). The 25 mg/kg dose (97% inhibition of the area under the ankle thickness vehicle curve) showed comparable efficacy to dexamethasone. In naïve rats treated with vehicle, the ankle diameters did not change over the course of the study and ankles from normal rats were significantly different ($P < 0.05$) compared with the CIA rats treated with vehicle. In

addition, **G-744** was also highly efficacious in preventing IFNα-driven lupus nephritis.[24]

Figure 2: Rat CIA Study - Treatment with **G-744** protects Lewis rats from collagen-induced arthritis. Female Lewis rats (n = 10 per group) with developing CIA were dosed orally with **G-744** as indicated (b.i.d.) or 0.05 mg/kg dexamethasone daily starting on day 0. Daily ankle diameter measurements are shown as mean ± SEM and were significantly (by ANOVA) reduced toward normal for all drug-treated rats (significant days 10-17) as compared to the vehicle control.



In summary, orally bioavailable Btk inhibitors with novel tricyclic head groups were discovered through structure and property based drug design. Improved molecules were discovered and an outstanding tool molecule, **G-744**, was identified with excellent potency, favorable DMPK properties and superb kinase selectivity. **G-744** demonstrated efficacy equivalent to Dexamethasone in a rat CIA model at 25 mg/kg b.i.d. dosing. Such findings further solidified our commitment to Btk as a therapeutic target. The chemistry culminating in a clinical candidate will be described in a subsequent manuscript.

ASSOCIATED CONTENT

Supporting Information (SI). Physiochemical properties, experimental procedures, compound characterization, assay protocols, PK-PD relationship, and full kinome data are included. This information is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

Btk, Bruton's tyrosine kinase; RA, Rheumatoid arthritis; SLE, systemic lupus erythematosus; MS, Multiple sclerosis; MCL, Mantle cell lymphoma; CLL, chronic lymphocytic leukemia; BCR, B-cell receptor; DMPK, Drug metabolism and pharmacokinetics; CIA, collagen-induced arthritis; b.i.d., twice a day. SEM: standard error of the mean.

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Discovery of Potent and Selective Tricyclic Inhibitors of Bruton's Tyrosine Kinase with Improved Drug-like Properties

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